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UNITED STATES PATENT APPLICATION

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FOR: ROLLER BOTTLE

RELATED APPLICATIONS

[0001] This application claims priority from U.S. Provisional Patent Application Serial No. 60/437,748, filed on January 2, 2003 and U.S. Provisional Patent Application Serial No. 60/505,742, filed on September 25, 2003.

BACKGROUND OF THE INVENTION

1. Field of the Invention

[0002] This invention relates to a container for cell culture production, and more particularly to a roller bottle having a helical pleat array for achieving a high cell growth surface area and for facilitating access to all surface areas by liquids deposited in the bottle.

2. Description of Related Art

[0003] One type of container commonly used in the laboratory for culturing cells is known as a "roller bottle". Roller bottles are generally cylindrical and are adapted to rotate about their axes. The internal surfaces of such roller bottles provide active surfaces for cells.

[0004] A liquid growth medium is introduced into the roller bottle. The rotating movement of the bottle keeps the internal surfaces wetted with a liquid medium, thereby encouraging the growth of cells.

[0005] Rotating rollers of an appropriate apparatus are employed to rotate these roller bottles. It is desirable to grow large amounts of cells, mostly for cell by-products, such as pharmaceutical substances that are secreted by cells. Various approaches have been used to increase the internal surface area of roller bottles. One approach has been to increase the amount of actual surface area available for cells to grow on.

[0006] Some roller bottles are produced unitarily by a blow-molding technique and include longitudinal pleats in the walls of the roller bottle. The longitudinal pleats increase the effective internal surface area of the roller bottle, and extend into the growth chamber for increasing culture or cell yield.

[0007] It is further known to provide a culture vessel having such longitudinal pleats, and further including circumferential collars that encircle the external surface of the top and bottom ends of the vessel. The collars maximize the vessel's grip point when the vessel is placed on its side on the rollers of a rotating apparatus.

[0008] The prior art also discloses a roller bottle having pleats cross-wise to the axis of the bottle for increasing the surface area for growing cells and further including at least one unpleated longitudinal drain panel. Axial reinforcing ribs extend along the outer edge of the cross-wise corrugation for strengthening the pleated bottle.

[0009] It is also known to use circumferential ribs on a roller bottle for reinforcing the bottle walls. In this regard, the prior art discloses a roller bottle having flexible plastic walls and a plurality of spaced-apart circumferential reinforcement rings defined in the flexible plastic walls to cause the body to retain a generally cylindrical shape.

[00010] A problem associated with prior art roller bottles, especially those having pleats for expanding the surface area for growing cells, has been the tendency of the bottle walls to expand when the insides of the bottles become pressurized. This expansion causes the bottle to stop rolling on its roller rack. The absence of rotation causes portions of the internal surfaces to become dry and promotes cell death in these dry areas.

[0010] Another problem associated with prior art roller bottles relates to the ability to assure that liquid placed in the roller bottle will achieve maximum contact with all surface regions of the pleated walls. In this regard, it has been determined that roller bottles with circumferential pleats will distribute liquid efficiently within any given pleat as the roller bottle is rotated about its axis. However, liquid may not be distributed uniformly among the various circumferential pleats. Longitudinal pleats allow liquid to flow longitudinally along the grooves defined by the pleats. However, rotation of the roller bottle about its axis does not always distribute liquid efficiently from one pleat to another. A roller bottle with some circumferential pleats and some longitudinal pleats can achieve better distribution of liquids than a roller bottle that relies only upon longitudinal pleats or circumferential pleats.

[0011] It is desirable therefore to provide a pleated roller bottle which is reinforced so as to add rigidity to the vessel wall to prevent the bottle from expanding to the point where the roller bottle stops rolling.

[0012] It is also desirable to provide roller bottles with pleats that permit efficient flow of liquid to all surface areas.

SUMMARY OF THE INVENTION

[0013] The present invention provides a roller bottle for cell growth culturing including an elongate cylindrical wall having a closed bottom end and a liquid opening at an

opposing top end. The elongate cylindrical wall includes a helically extending pleat that extends from the closed end to the top end. The elongate cylindrical wall may further include a longitudinal flat or two diametrically opposed longitudinal flats that run the length of the bottle.

[0014] The helical or spiral pleat has an advantage over circumferential pleats when cells are harvested from the bottle. In particular, cells are collected from the bottle by first pouring off the media and then detaching the cells from the bottle walls. Frequently, a trypsin solution is poured into the bottle and then distributed around to cover all of the cells. A continuous spiral enables the trypsin to more easily reach all of the cells because the trypsin can follow a continuous path around the helix as the bottle is rotated about its axis. In contrast, circumferential pleats or axial pleats may complicate uniform distribution of trypsin to all areas of the bottle from which cells may be harvested. Thus, a much more thorough coating with trypsin is achieved with a helical pleat or an array of helical pleats.

DESCRIPTION OF THE DRAWINGS

[0015] FIG. 1 is a side elvational view of a roller bottle of the present invention.

[0016] FIG. 2 is a horizontal sectional view taken along line 2-2 of FIG. 1.

[0017] FIG. 3 is a cross-sectional view taken along line 3-3 in FIG. 2.

DETAILED DESCRIPTION

[0018] Referring to the drawings in which like reference characters refer to like parts throughout the several views thereof, the roller bottle **10** of the present invention is shown. Roller bottle **10** includes a cylindrical side wall **12** which extends from a bottom wall **14** to an opposing top wall **16**. Extending from top wall **16** and integral therewith is

a neck **18** having extended screw threads **20** for receiving an internally threaded screw cap (not shown) thereon in the usual manner. Other cap connections, such as bayonet connections, may be used. Neck **18** may include a locking arrangement **22** for holding a cap in a locked open position on the roller bottle for maintaining the roller bottle open to the environment surrounding it.

[0019] As described above, a problem associated with prior art roller bottles that have longitudinal pleats has been that the pleats tend to expand when the inside of the bottle becomes pressurized. In use, the roller bottle is sealed and warmed in an incubator. Warming raises the pressure inside the roller bottle, typically about 1 psi. The internal pressure in the roller bottle may bow the walls outward. This causes the bottle to stop rolling on the roller rack. As a result, portions of the interior surface of the bottles become dry, leading to cell death and a reduction in culture yield.

[0020] Roller bottle **10** addresses the problem of side wall expansion by providing a helical or spiral pleat **24** extending along cylindrical side wall **14** of roller bottle **10** from a location substantially adjacent bottom wall **14** to a location substantially adjacent top wall **16**. Pleat **24** preferably defines a small angular pitch "a" that preferably is between 2° and 10°, and not preferably about 5°. A small angular pitch "a" for helical pleat **24** provides a reinforcing effect similar to circumferential pleats, and additional reinforcing structures need not be molded into side wall **12** of bottle **10**. As can be seen in FIGS. 3 and 3, pleat **24** provides a plurality of opposed facing internal surfaces **26** for the formation of cell growth thereon. Thus, the pleated structure increases the active surface area internally of roller bottle **10** in comparison to a conventional flat wall structure.

[0021] While the present invention may be constructed in various sizes and configurations, the preferred structure of the present roller bottle includes a bottle which is approximately 27.08 cm (10.66 inches) in length from the top of neck **18** to the bottom

of base **14**. Such a configured bottle defines a growth surface area length of about 22.86 cm (9.0 inches) having a diameter of about 11.76 cm (4.63 inches). As shown in FIG. 3, the pleats forming facing internal surfaces **26** define a generally wave-like structure having outer and inner apices **28** and **30**, respectively. The distance between the outer apices **28** of two adjacent passes of helical pleat **24** is approximately 0.82 cm (0.323 inches). Facing internal surfaces **26** of adjacent passes of helical pleat **24** define an interior angle "b". In the present embodiment, angle "b" may be approximately 60°. It is noted that the apices **28** and **30** of pleat **24** are desirably rounded to facilitate cell adherence to the internal surfaces **26**. Moreover, such rounded surfaces are easy to form by casing or molding and are stronger and less subjective cracking upon flexing.

[0022] As shown in FIGS. 1-2, roller bottle **10** may further include diametrically opposed longitudinally extending planar sections **32** that presents an interruption of pleat **24** to enhance the microscopic viewing of the contents of roller bottle **10** and/or to facilitate the formation thereof. In preferred embodiments of the present invention, the roller bottle contains two diametrically opposed planar sections **32** to facilitate microscopic viewing. The particular arrangement of planar sections **32** shown in the drawings is not critical. Moreover, the width of planar sections **32** is not critical, except that these panels also represent a loss of increase surface area. A problem associated with prior art pleated roller bottles having planar sections for microscopic viewing has been that these sections become distorted when the inside of the bottle becomes pressurized, making microscopic viewing difficult.

[0023] It is noted that roller bottle **10** may include a recessed portion (not shown) at bottom wall **14** to facilitate stacking of the roller bottle with a similar bottle in a nesting relationship.

[0024] The present invention provides a helically pleated roller bottle that can be used to increase the culture or cell yields per unit for either adherent-type cells or cells that grow in suspension. It is noted that adherent-type cells include cells which require a support surface to grow on, as well as cells capable of growing on a support surface. By the phrase "increase in culture or cell yields", it is meant that there is an increase in the number of cells and/or their by-products per culture vessel or unit. As shown in the drawings herein, cylindrical side wall **12** of roller bottle **10** has a helical pleat **24** along a substantial portion of its length, thus corrugating the interior surfaces **26**. The interior pleating increases the surface area available for the attachment and growth of adherent-type cells, thus increasing the culture or cell yields per vessel. Moreover, it is noted that with respect to the cells which grow in suspension, the interior corrugating of roller bottle **10** enhances agitation of the suspension culture and helps disperse the cells and promote growth of the culture.

[0025] After cells have formed on internal surfaces **26** of roller bottle **10** by rolling in the proper environment for the formation of the cells, roller bottle **10** with formed cells on the walls thereof is removed from the convention roller bottle apparatus. The cell forming liquid media remaining in bottle **10** may be decanted from bottle **10** along helical pleat **24** and flat viewing panels **32** and a small amount of saline solution may be added to prevent the cells from drying. Alternatively, the liquid media may remain if it is only a small amount. Thereafter, a scraper apparatus may be used for removing cells from the internal surfaces **26** of roller bottle **10**.

[0026] A more conventional procedure for removing cells is the introducing of a solution containing the proteolytic enzyme trypsin, together with a chelating agent, which has the effect of causing the cells to release from internal surfaces **26** for decanting from roller bottle **10** along helical pleat **24** and longitudinal planar sections **32**. More

particularly, helical pleat **24** ensures that the trypsin solution follows the helical path formed by pleat **24** from one end of bottle **10** to the other merely by rotating bottle **10** about its longitudinal axis **34**. Hence, complete exposure to trypsin solution is assured, thereby maximizing cell yield. Subsequently, bottle **10** generally is discarded. Thus, the advantage of providing a roller bottle, such as that of the present invention, which may be inexpensively produced by blow-molding, used once and discarded is readily apparent. The inventive roller bottle provides a unitary structure including an elongate cylindrical wall with a helical pleat that provides a greatly increased surface area for cell growth formation therein. The helical pleat on the cylindrical wall further reinforce the bottle walls and eliminate the need to discard the roller bottle prematurely during culturing due to repeated flexing of the pleats which can cause fatigue and cause the bottle to stop rolling on the roller bottle apparatus, especially during long periods of use during the cell culturing batch process. Helical pleat **24** prevents the bending and extension of side wall **12**.

[0027] In viewing generally the conditions for producing roller bottles in accordance with the invention, a variety of thermoplastic materials maybe utilized including, for example polystyrene, polyethylene terephthalate, the polyolefins and polyvinyl chloride. Polystyrene is particularly desirable as cells appear to grow well and in large numbers on this material.

[0028] The wall of the bottle should have a sufficient thickness to provide a bottle with adequate strength when filled with liquid medium. Typically, the film thickness will be from 1-60 thousandths of an inch for a 2.25 liter roller bottle. The thermoplastic resin used for forming bottles by extrusion, blow or injection blow-molding techniques should be able to readily flow to form the helical pleat.

[0029] It is noted that only preferred embodiments of the invention have been described and that numerous substitutions, modifications and alterations are permissible without departing from the spirit and scope of the invention.